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Degradation of Vinyl Acetate by Soil, Sewage, Sludge, and the Newly Isolated Aerobic Bacterium V2

M. NIEDER, B. SUNARKO, AND O. MEYER*

Lehrstuhl für Mikrobiologie der Universität Bayreuth, Universitätsstrasse 30, D-8580 Bayreuth, Federal Republic of Germany

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Vinyl acetate is subject to microbial degradation in the environment and by pure cultures. It was hydrolyzed by samples of soil, sludge, and sewage at rates of up to 6.38 and 1 mmol/h per g (dry weight) under aerobic and anaerobic conditions, respectively. Four yeasts and thirteen bacteria that feed aerobically on vinyl acetate were isolated. The pathway of vinyl acetate degradation was studied in bacterium V2. Vinyl acetate was degraded to acetate as follows: vinyl acetate + $NAD(P)^+ \rightarrow$ 2 acetate + NAD(P)H + H^+. The acetate was then converted to acetyl coenzyme A and oxidized through the tricarboxylic acid cycle and the glyoxylate bypass. The key enzyme of the pathway is vinyl acetate esterase, which hydrolyzed the ester to acetate and vinyl alcohol. The latter isomerized spontaneously to acetaldehyde and was then converted to acetate. The acetaldehyde was disproportionated into ethanol and acetate. The enzymes involved in the metabolism of vinyl acetate were studied in extracts. Vinyl acetate esterase (K_m = 6.13 mM) was also active with indoxyl acetate (K_m = 0.98 mM), providing the basis for a convenient spectrophotometric test. Substrates of aldehyde dehydrogenase were formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde. The enzyme was equally active with NAD^+ or $NADP^+$. Alcohol dehydrogenase was active with ethanol (K_m = 0.24 mM), 1-propanol (K_m = 0.34 mM), and 1-butanol (K_m = 0.16 mM) and was linked to NAD^+. The molecular sizes of aldehyde dehydrogenase and alcohol dehydrogenase were 145 and 215 kilodaltons, respectively.

Vinyl acetate (acetic acid ethenyl ester, $CH_3CO_2CH{:}CH_2$) is a monocarboxylic, unsaturated aliphatic ester which will react violently with itself to yield polyvinyl acetate. Because of this, it is shipped with an inhibitor (3 to 20 μg of hydroquinone or diphenylamine per liter) to prevent polymerization (10, 15). Vinyl acetate is a clear, colorless liquid which can form flammable and explosive mixtures with air. Its solubility in water is only 1.98% (wt/vol) at 20°C (10). Subjective response studies indicate that the sweetish smell of vinyl acetate is detectable at levels as low as 0.4 μg/liter, with no significant eye or upper respiratory irritation occurring below 10 μg/liter (5). The industrial use of vinyl acetate is for the production of polyvinyl acetate, polyvinyl alcohol, and other polymers or copolymers. Its worldwide annual production capacity was about 3 million tons in 1982 and is still increasing (15).

In a study examining the oxidation of several vinyl compounds by sewage under aerobic conditions, vinyl acetate was converted to CO_2, indicating its susceptibility to biological degradation (13). The anaerobic conversion of vinyl acetate to methane by municipal sludge has also been shown (21). Fungi can grow in association with ethylene-vinyl acetate co-polymers (6), and polyvinyl acetate is degraded by strains of *Aspergillus* and *Penicillium* spp. (23). Activity towards vinyl acetate has not been reported, however. In higher organisms, vinyl acetate was enzymatically hydrolyzed to acetaldehyde and acetate (18), and no acetoxyoxirane (the epoxide of vinyl acetate) was formed (17).

Since we were not aware of studies dealing with the microbial metabolism of vinyl acetate in pure cultures, we have examined environmental samples for activity towards vinyl acetate, isolated active microorganisms, and characterized the metabolism of vinyl acetate by the bacterium V2 in greater detail.

* Corresponding author.

MATERIALS AND METHODS

Media and growth conditions. The mineral medium of Schlegel et al. (16) was used throughout, with the modification that phosphates were reduced to one third. The medium was supplied with 1 ml of trace element solution TS2 (11) per liter reduced in sodium molybdate (0.03 g/liter). The pH was adjusted to 7.2. If not otherwise indicated, media were provided with 20 mM of the organic substrate. Media were solidified with 1.5% (wt/vol) agar (Difco Laboratories, Detroit, Mich.). Vinyl acetate was supplied by evaporation from soaked filter paper attached to the inner side of the top dish. Petri dishes were kept upside down in taped plastic bags. Growth was at 30°C. Growth experiments under aerobic conditions were carried out in shaken 1-liter Erlenmeyer flasks containing 500 ml of mineral medium supplied with vinyl acetate, acetate, ethanol, or acetaldehyde as the sole sources of carbon and energy.

Anaerobic growth was checked in 50-ml Erlenmeyer flasks containing 20 ml of the media indicated above. The flasks were kept shaken in desiccators and made anaerobic by using the GasPak anaerobic system (E. Merck AG, Darmstadt, Federal Republic of Germany). Growth was followed by A_{436} measurements in a spectrophotometer (Ultrospec 4050; LKB, Freiburg, Federal Republic of Germany).

Enrichment cultures. Five samples of humus-rich garden soil (pH 6.9 to 7.4) and sewage (see below) were collected, and 0.5 g or 0.5 ml of each of these, respectively, was added to 50-ml Erlenmeyer flasks containing 15 ml of mineral medium supplied with 20 mM vinyl acetate. Flasks were kept shaken at 30°C in desiccators filled with air. After 1 week, the first subcultures were made (10% [vol/vol] inoculum), and growth usually occurred after 1 day. Portions of the cultures were streaked on mineral plates and incubated

in vinyl acetate vapor. Emerging single colonies were restreaked and tested for the utilization of vinyl acetate. Finally, the uniformity of colonies was examined by restreaking on 0.5% yeast extract–agar. Uniformity of cell shape was checked by microscopy.

Degradation of vinyl acetate by environmental samples. Samples of loamy soil from a cornfield (0.1 g [wet weight], pH 6), combined aerobic sewage (4 ml, pH 7.4, 1.7 g [dry weight] per liter), or combined anaerobic sludge (2 ml, pH 7, 29.6 g [dry weight] per liter) from the local wastewater treatment plant were made up to 5 ml with 50 mM KH_2PO_4-NaOH buffer, pH 7.2, and incubated in serum-stoppered vials of 60 ml (total volume). Vinyl acetate (20 mM final concentration) was injected by using a syringe. Aerobic incubation was under air. Anaerobiosis was established by sparging the flasks with N_2 prior to the addition of vinyl acetate. Control assays were autoclaved for 20 min at 121°C prior to the addition of vinyl acetate. All flasks were kept shaken at 30°C. Samples (245 μl) were removed over time by means of a syringe and mixed with 5 μl of aqueous 2 M HCl to stop biological activities. Assays were subsequently clarified by low-speed centrifugation and analyzed with a gas chromatograph for the presence of vinyl acetate, acetate, acetaldehyde, and ethanol.

Activities of resting bacteria towards vinyl acetate and its metabolic derivatives. Resting cell suspensions of vinyl acetate-grown isolate V2 were prepared as follows. Exponential cells were washed by low-speed centrifugation in phosphate buffer and kept at −20°C until use. One gram of frozen cell paste was suspended in 0.3 ml of phosphate buffer before use. Of this suspension, 100-μl volumes were injected into serum-stoppered vials, incubated, and analyzed as described above for the degradation of vinyl acetate by environmental samples. Acetaldehyde, ethanol, and acetate were also tested.

Gas chromatographic analyses of metabolites. Consumption or production of vinyl acetate, acetate, ethanol, and acetaldehyde were measured in a gas chromatograph (model 430; Packard, Müllheim, Federal Republic of Germany) equipped with a flame ionization detector and a recorder (model 641; Packard). Samples (1 μl) were withdrawn from assays by use of a syringe and injected into the gas chromatograph operated under the following conditions: glass columns (0.25 in. [ca. 0.64 cm] by 3 m) filled with Porapak Q (80 to 100 mesh); carrier gas, N_2 at a flow rate of 11 ml/min; oven temperature, 200°C; injection port and detector temperature, 240°C. Under these conditions the detection limits were better than 1 μmol/ml.

Preparation of bacterial extracts. Suspensions of washed bacteria (ca. 40 g [wet weight]) in phosphate buffer, (about 40 ml) pH 7.5, were supplied with a few crystals of DNase I and passed four times through a precooled French pressure cell (American Instrument Co., Silver Spring, Md.) operated at maximum pressure. The resulting crude bacterial extract was centrifuged at 11,400 × *g* for 40 min (rotor JA 20; Beckman Instruments, München, Federal Republic of Germany) to remove unbroken cells and cell debris. Subsequently, the supernatant was subjected to ultracentrifugation at 203,400 × *g* for 3 h (Centricon T-1065, rotor TFT 45.94; Kontron, Eching, Federal Republic of Germany) yielding a supernatant and a pellet, designated cytoplasm and cytoplasmic membranes, respectively. Membranes were suspended in phosphate buffer before use.

Enzyme assays. Enzyme activities were determined spectrophotometrically (model UV-120-02; Shimadzu, Kyoto, Japan) at 30°C.

Vinyl acetate esterase (EC 3.1.1.6). Since bacterial extracts contained very active NAD-dependent alcohol dehydrogenase and acetaldehyde dehydrogenase, the hydrolysis of vinyl acetate could be coupled to the reduction of NAD^+ by these enzymes. Cuvettes were filled with 1 ml of phosphate buffer, pH 7.2, containing 10 mM vinyl acetate and 3 mM NAD^+, and reactions were started by injecting extract (usually 30 μl). The formation of NADH + H^+ was followed at 365 nm. Activities were calculated assuming that 1 mol of NADH + H^+ was formed per mol of vinyl acetate hydrolyzed.

Routinely, vinyl acetate esterase was assayed spectrophotometrically by measuring the formation of indoxyl from indoxyl acetate. To a cuvette containing 0.9 ml of phosphate buffer, pH 7.2, 0.1 ml of an indoxyl acetate stock solution (100 mM in ethanol) was added. Reactions were started with extract as above. The increase of A_{375} was followed. The millimolar extinction coefficient of indoxyl was taken as 2.54/mmol per cm.

Alcohol dehydrogenase [alcohol:NAD(P)$^+$ oxidoreductase, EC 1.1.1.71] and aldehyde dehydrogenase [aldehyde:NAD(P)$^+$ oxidoreductase, EC 1.2.1.5]. Assays for alcohol dehydrogenase and aldehyde dehydrogenase contained 3 mM NAD^+ or $NADP^+$ and 1 mM alcohol or aldehyde in 1 ml of phosphate buffer, pH 7.5. Reactions were started by adding up to 10 μl of extract. The formation of NAD(P)H + H^+ was followed at 365 nm.

Acetyl coenzyme A (acetyl-CoA) synthetase (EC 6.2.1.1) was measured in a coupled assay with citrate synthase and malate dehydrogenase as described previously (24).

Isocitrate lyase (EC 4.1.3.1) was assayed by measuring the formation of glyoxylic acid phenylhydrazone from glyoxlate (G. H. Dixon and H. L. Kornberg, Biochem. J. **72**:3P, 1959).

Oxidase (8) and catalase (19) were assayed on bacterial colonies as described previously.

Gram staining was done by the protocol of Bartholomew (1).

Protein estimation. The method of Bradford (4) was employed for protein determination.

Determination of molecular size. Apparent molecular sizes of alcohol dehydrogenase and aldehyde dehydrogenase were determined by subjecting cytoplasmic fractions to gel filtration on Sepharose CL 6B (Pharmacia-LKB, Freiburg, Federal Republic of Germany) by using the following standards (Molecular sizes in parentheses): RNase A (13.7 kilodaltons [kDa]), chymotrypsinogen A (25 kDa), ovalbumin (43 kDa), albumin (67 kDa), aldolase (158 kDa), catalase (232 kDa), ferritin (440 kDa), and thyroglobulin (669 kDa). Column size and bed volume were 2.6 by 100 cm and 500 ml, respectively.

Chemicals. Vinyl acetate and indoxyl acetate were from Fluka (Neu-Ulm, Federal Republic of Germany), and acetaldehyde was from Merck. All other chemicals were purchased from commercial sources.

RESULTS

Degradation of vinyl acetate by soil, sewage, and sludge samples. All samples of soil, sewage, and sludge actively degraded vinyl acetate under the conditions employed (Fig. 1). Vinyl acetate degradation was observed under aerobic and anaerobic conditions and led to the formation of stoichiometric amounts of acetate and acetaldehyde. Ethanol was not formed under any of the conditions employed, and accumulation of intermediates was presumably the result of nutrient limitation. Autoclaved controls showed only slight formation of acetate or acetaldehyde because of some non-

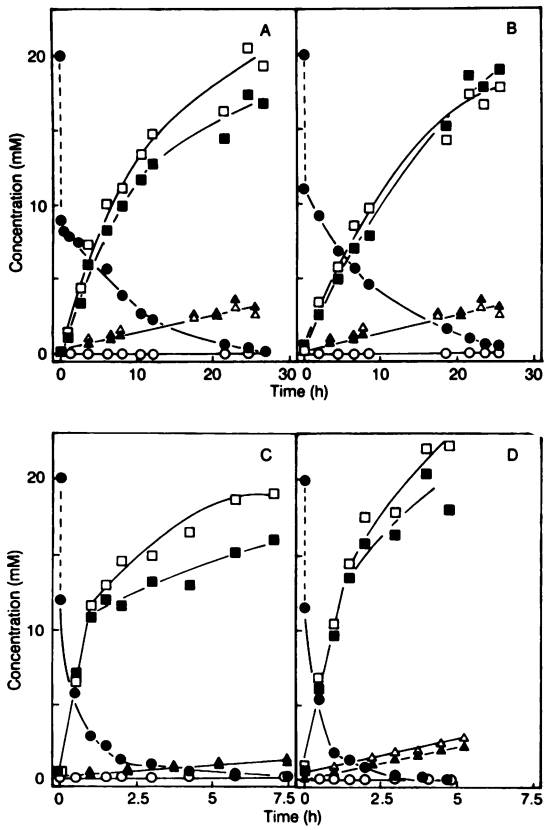

FIG. 1. Degradation of vinyl acetate by soil, sewage, and sludge samples. The formation of acetate (□), acetaldehyde (■), and ethanol (○) from vinyl acetate (●) in samples of soil (A, B), sewage (C), and sludge (D) under air (A, C) or N_2 (B, D) was followed with a gas chromatograph as detailed in Materials and Methods. The formation of acetate (△) or acetaldehyde (▲) was also examined in autoclaved controls.

biological hydrolysis of vinyl acetate that occurred (Fig. 1). The sharp decrease of the vinyl acetate concentration within the first 30 min of incubation (Fig. 1) indicates some adsorption of the compound to the samples. However, even the adsorbed vinyl acetate was accessible to microbial hydrolysis. Hydrolyzing activities of soil or sewage samples under aerobic conditions ranged from 0.09 to 6.38 mmol of vinyl acetate per h per g [dry weight]. With soil or sludge samples under anaerobic conditions, hydrolyzing activities ranged from 0.11 to 1 mmol of vinyl acetate per h per g [dry weight].

Enrichment and isolation. Microorganisms feeding on vinyl acetate were isolated from enrichment cultures inoculated with various samples of soil and sewage, as described in Materials and Methods. Pure cultures of four yeasts (V14 through V17) and 13 bacteria (V1 through V13) were obtained. All isolates could grow on vinyl acetate as the sole source of carbon and energy and were preliminarily characterized. Most bacteria were short and thick or long and thin rods. Colonies were colorless, except for isolate V9 which was yellow. The isolates V8, V11, and V12 stained gram positive; all others were gram negative. The isolates V1 and V13 were oxidase positive. The isolates V2, V7, and V8 were catalase negative.

Since the cells of isolate V2 grew rapidly as a homogeneous suspension in liquid media, this bacterium was chosen for further studies.

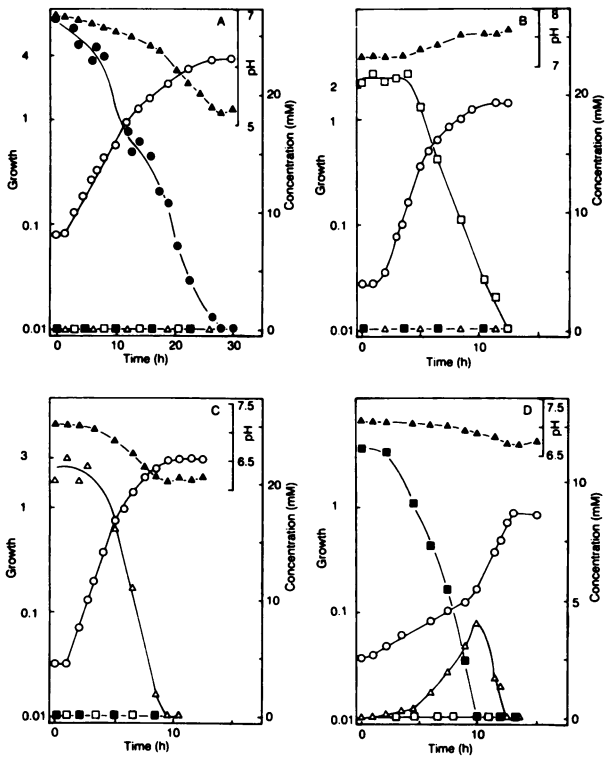

FIG. 2. Growth of bacterium V2 on vinyl acetate and its metabolic derivatives. Growth was examined in 500-ml cultures supplied with vinyl acetate (A), acetate (B), ethanol (C), and acetaldehyde (D) as described in Materials and Methods. Symbols: ○, growth at A_{436}; ●, vinyl acetate; □ acetate; ■, acetaldehyde; △, ethanol; ▲, pH.

Properties of isolate V2. The cells of strain V2 were gram negative straight rods with a width of 1.2 to 1.6 μm and a length of 1.6 to 5.2 μm. Bacteria were nonflagellated, nonmotile, and did not form endospores. They were oxidase and catalase negative and had an aerobic respiratory metabolism. No visible pigments were formed under any conditions. Besides vinyl acetate, growth was also supported by acetate, ethanol, acetaldehyde, pyruvate, and complex substrates. Methyl acetate, ethyl acetate, propyl acetate, butyl acetate, vinyl chloride, glucose, fructose, and citrate were not utilized.

Growth on vinyl acetate. Isolate V2 could grow aerobically in a medium containing vinyl acetate as the sole source of carbon and energy (Fig. 2A). Under these conditions, doubling times of 3 h and final optical densities of about 4 were obtained. Intermediates of vinyl acetate hydrolysis, such as acetate, ethanol, or acetaldehyde (see below), did not appear in the culture broth (Fig. 2A). Because of the assimilation of ammonia, growth was accompanied by a drop in pH.

Vinyl acetate is also subject to nonenzymatic hydrolysis. Its half-life in sterile medium was about 60 h and decreased to 12 h through the action of growing bacteria (Fig. 2A).

Growth on acetate, ethanol, and acetaldehyde. Growth of isolate V2 with acetate (doubling time [t_d] = 0.9 h) or ethanol (t_d = 0.8 h) was much faster than with vinyl acetate (Fig. 2). During growth with these substrates, no intermediates could be detected in the medium. Utilization of acetate or ethanol led to an increase or a decrease, respectively, of the pH in the culture (Fig. 2).

Growth of isolate V2 on acetaldehyde was biphasic (Fig.

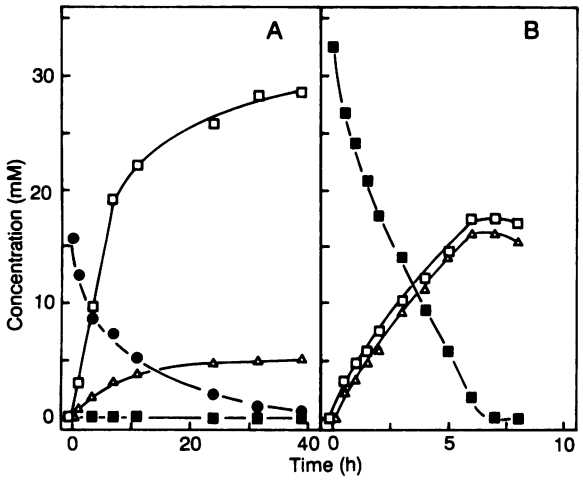

FIG. 3. Cleavage of vinyl acetate (A) and disproportionation of acetaldehyde (B) by resting bacteria. The formation of products in resting cell suspensions under N_2 was examined as described in Material and Methods. Symbols: ●, vinyl acetate; □, acetate; ■, acetaldehyde; △, ethanol.

2D). The corresponding doubling times were 4.8 and 1.3 h, respectively. During the first growth phase, about 60% of the acetaldehyde was utilized for growth and the remaining 40% was converted to ethanol and excreted into the medium (Fig. 2D). The second growth phase was characterized by the utilization of ethanol, which obviously is a much better substrate than acetaldehyde (it is certainly less toxic). Only negligible amounts of acetate were formed under the conditions examined in Fig. 2.

Apparent millimolar growth yields (A_{436}/mmol) of isolate V2 were determined with vinyl acetate (0.143), acetaldehyde (0.08), ethanol (0.142), and acetate (0.068).

Intermediates of vinyl acetate metabolism. The metabolism of vinyl acetate was examined under conditions which did not allow complete oxidation but led to the excretion of intermediates into the culture broth (Fig. 3). For this purpose, resting cell suspensions of isolate V2 were incubated with vinyl acetate or acetaldehyde under N_2. At low concentrations (around 15 mM), vinyl acetate was stoichiometrically converted to ethanol and acetate (Fig. 3A; Table 1). At high concentrations (around 30 mM), in addition to ethanol and acetate, acetaldehyde also appeared (Table 1). Similar results were obtained with resting cell suspensions in the presence of air (Table 1). Anaerobic suspensions of resting bacteria disproportionated acetaldehyde quantitatively to ethanol and acetate at a 1:1 molar ratio (Fig. 3B). The experiments with vinyl acetate and acetaldehyde also showed that resting cell suspensions of strain V2 cannot metabolize ethanol and acetate.

TABLE 1. Stoichiometry of products formed from vinyl acetate by resting cells of bacterium V2

Concn of substrate (vinyl acetate) (mM)[a]	Concn of product (mM)		
	Acetate	Ethanol	Acetaldehyde
18.0 (under N_2)	33.3	5.6	0
14.5 (under air)	23.0	4.3	0
30.8 (under N_2)	45.5	8.5	8.2
33.1 (under air)	37.5	9.4	10.8

[a] For details of the conditions, see Materials and Methods.

TABLE 2. Distribution of enzyme activities related to vinyl acetate metabolism in subcellular fractions of bacterium V2

Subcellular fraction	Amt of protein (mg)	Total activity (μmol/min)[a]		
		Vinyl acetate esterase	Alcohol dehydrogenase	Aldehyde dehydrogenase
Crude extract	2,646	131 (100)	5,678 (100)	3,402 (100)
Cytoplasm	2,099	120 (92)	5,850 (103)	3,256 (96)
Cytoplasmic membranes	49	1 (1)	38 (1)	46 (1)

[a] Percentages are indicated in parentheses.

Subcellular location of enzymes involved in vinyl acetate metabolism. Activities of vinyl acetate esterase, alcohol dehydrogenase, and aldehyde dehydrogenase were stable in subcellular fractions of isolate V2 grown on vinyl acetate and appeared quantitatively in the cytoplasmic fraction (Table 2).

Activities and other properties of enzymes involved in the metabolism of vinyl acetate. We were able to demonstrate in cytoplasmic fractions of isolate V2 enzyme activities towards vinyl acetate and the derivatives of its metabolism suggested by the above experiments with growing and resting bacteria (Table 3). In addition to these, enzyme activities characteristic of the formation of acetyl-CoA from acetate and the glyoxylate bypass could also be demonstrated (Table 3).

Vinyl acetate esterase was also active with indoxyl acetate (Table 3), yielding acetate and yellow-colored indoxyl. The enzymatic reaction was completed before onset of the spontaneous indigo blue formation under the influence of O_2 and thus provided the basis for a convenient photometric test of enzyme activity. In addition, the enzyme was specific for vinyl acetate and had less than 3% activity with acetic acid esters of other C_1 to C_4 aliphatic alcohols. The K_m values for vinyl acetate hydrolysis by microsomes, plasma (rat or human), purified acetylcholine esterase, butyrylcholine esterase, or carboxyl esterase ranged from 0.65 to 77 mM (18). The affinity of vinyl acetate esterase from bacterium V2 favorably compared with these figures (Table 3).

TABLE 3. Activities, specificities, and some kinetic properties of enzymes in cytoplasmic fractions of bacterium V2

Enzyme or substrate	Sp act (μmol/min per mg of protein)	K_m (mM)	V_{max} (μmol/min per mg of protein)
Esterase			
Vinyl acetate	0.10	6.13	0.19
Indoxyl acetate	0.13	0.98	0.14
Alcohol dehydrogenase			
Ethanol	2.83	0.24	3.95
1-Propanol	3.30	0.34	5.08
1-Butanol	1.24	0.16	1.72
Aldehyde dehydrogenase			
Formaldehyde	3.31	1.28	7.94
Acetaldehyde	3.36	NL[a]	NL
Propionaldehyde	2.67	NL	NL
Butyraldehyde	0.96	NL	NL
Acetyl-CoA synthetase	0.05	ND[b]	ND
Isocitrate lyase	0.47	ND	ND

[a] NL, No Lineweaver-Burk kinetics.
[b] ND, Not determined.

Alcohol dehydrogenase was active with C_2 to C_4 aliphatic alcohols (Table 3). It was inactive with methanol. The enzyme was linked to pyridine nucleotides with a preference for NAD^+. Its activity with ethanol and $NADP^+$ was only 4% of that with NAD^+. The reaction did not require the addition of semicarbazide, which was even inhibitory. Alcohol dehydrogenase could also catalyze the reduction of acetaldehyde to ethanol in the presence of NADH + H^+. Its affinity for ethanol was very high (Table 3) compared with alcohol dehydrogenases from *Alcaligenes eutrophus* (5 mM) (20), *Acetobacter polyoxogenes* (1.2 mM) (22), *Leuconostoc mesenteroides* (71 mM) (2), *Zymomonas mobilis* (1.7 and 100 mM) (25), and *Schwanniomyces castellii* (3.5 mM) (12). The apparent molecular size of alcohol dehydrogenase as determined by gel filtration was 215 kDa.

Aldehyde dehydrogenase (oxidizing) was active with C_1 to C_4 aliphatic aldehydes, yielding the corresponding acids (Table 3). When using acetaldehyde as the substrate, the enzyme was equally active with NAD^+ and $NADP^+$. The specific activity of the reduction of acetate to acetaldehyde was 8% of that of the forward reaction. Gel filtration yielded an apparent molecular size of 145 kDa for aldehyde dehydrogenase.

Vinyl acetate esterase was perfectly stable in cytoplasmic fractions stored at 4°C. In contrast, alcohol dehydrogenase and aldehyde dehydrogenase were inactivated, with a half-life of about 2 days, when kept at 4°C in phosphate buffer containing 0.01% azide to prevent bacterial growth. Inactivation was less than 10% after 2 weeks of incubation at 4°C in the presence of 30% (vol/vol) glycerol.

DISCUSSION

Vinyl acetate is subject to microbial degradation in the environment. The microflora inhabiting soil, sewage, and sludge are capable of hydrolyzing vinyl acetate without lag (Fig. 1). The reaction occurred under aerobic as well as under anaerobic conditions, although hydrolysis was considerably faster in the presence of air. Regardless of the conditions, the products were acetaldehyde and acetate.

Vinyl acetate is utilized by pure cultures of newly isolated microorganisms. The isolation of vinyl acetate-degrading microorganisms from their natural habitats is convenient and poses no special problems. As exemplified by the 17 isolates obtained, utilization of vinyl acetate is widespread among bacteria of different taxonomic position and also occurs in yeasts. The microbial use of vinyl acetate as the sole source of carbon and energy described here represents a new metabolic type.

Stoichiometry of vinyl acetate utilization. The pattern of products formed from vinyl acetate by resting cell suspensions of bacterium V2 was determined by the concentration of vinyl acetate. At low concentrations, acetaldehyde was not formed, and the data of Table 1 support the following equation: 2 vinyl acetate → 3.4 acetate + 0.6 ethanol. At elevated concentrations vinyl acetate was hydrolyzed as follows (Table 1): 2 vinyl acetate → 2.6 acetate + 0.6 ethanol + 0.6 acetaldehyde. In growing bacteria, ethanol and acetaldehyde did not accumulate (Fig. 2) but were completely oxidized to acetate: vinyl acetate → 2 acetate.

Proposed pathway for the metabolism of vinyl acetate. On the basis of the results obtained in the present study, we propose a pathway for the conversion of vinyl acetate to acetate in bacterium V2 (Fig. 4). Important consequences of this pathway are the complete conversion of 1 vinyl acetate to 2 acetates with the concomitant generation of one

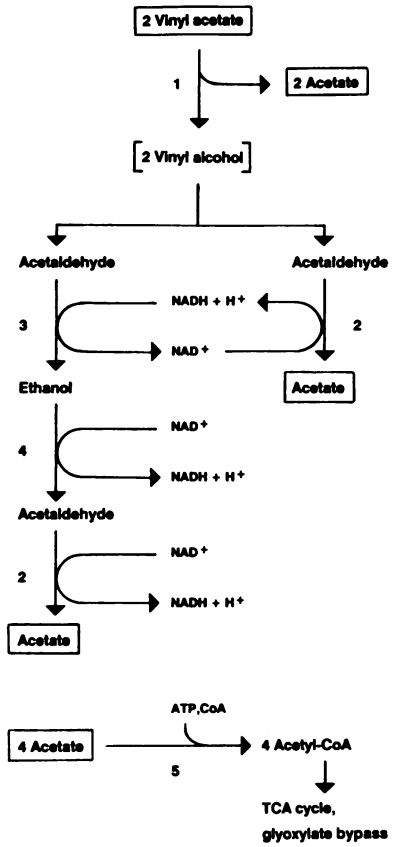

FIG. 4. Proposed pathway of vinyl acetate metabolism in bacterium V2. Enzymes involved are vinyl acetate esterase (1), aldehyde dehydrogenase (2), alcohol dehydrogenase (3, 4), and acetyl-CoA synthase (5). TCA, Tricarboxylic acid.

NAD(P)H + H^+. The initial reaction of this pathway is the hydrolysis of one molecule of vinyl acetate to acetate and acetaldehyde. The aldehyde is disproportionated into acetate and ethanol. The latter is subsequently oxidized via acetaldehyde to acetate. It is interesting to note that methyl acetate is cleaved by aerobic methylotrophic bacteria into acetate and methanol (14). As with bacterium V2, the acetate is assimilated through the tricarboxylic acid cycle and glyoxylate bypass. Depending on the organism, either methanol dehydrogenase and the ribulose monophosphate cycle or alcohol oxidase and the serine pathway are employed for the assimilation of methanol (14). Esterase activities towards naphthyl and nitrophenyl esters of fatty acids have been described in the anaerobic bacterium *Butyrivibrio fibrisolvens* (9). Esterase activity was also observed in other ruminal bacteria, e.g., *Bacteroides ruminicola*, *Selenomonas ruminantium*, *Ruminobacter amylophilus*, and *Streptococcus bovis*. Acetyl xylan esterase is believed to play an important role in the overall digestion of forage by these bacteria.

That the acid as well as the alcohol component of vinyl acetate are metabolized via acetate is supported by the following considerations. First, the bacterium contained a new vinyl acetate esterase capable of cleaving vinyl acetate into acetate and acetaldehyde. That these two compounds are actually intermediates of growing bacteria is obvious from their use as growth substrates and their stoichiometric excretion into the medium by resting cells. Although we

have not provided direct proof, it seems safe to assume that vinyl alcohol is the first product of vinyl acetate hydrolysis, especially since vinyl alcohol is not stable in aqueous systems and spontaneously isomerizes to acetaldehyde (3). Second, NAD^+-linked alcohol dehydrogenase and acetaldehyde dehydrogenase were present in bacterium V2. The enzymes catalyzed the disproportionation of two molecules of acetaldehyde to ethanol and acetate with no net production or consumption of reducing equivalents. This is further substantiated by the formation of stoichiometric amounts of acetate and ethanol from acetaldehyde by resting cells of bacterium V2 (Fig. 3B). In addition, acetaldehyde, ethanol, and acetate could serve as growth substrates (Fig. 2). Acetaldehyde was toxic for bacterium V2, since it impaired growth (Fig. 2D). Its conversion to ethanol and acetate would have the advantage of maintaining a low intracellular concentration of acetaldehyde. Another advantage of the disproportionation of acetaldehyde lies in the immediate generation of reduced pyridine nucleotides through acetate formation by aldehyde dehydrogenase to initiate ethanol synthesis. Third, bacterium V2 contained active alcohol and acetaldehyde dehydrogenases for the conversion of ethanol to acetate (Table 3). Both compounds could serve as growth substrates (Fig. 2). Furthermore, we observed a temporary increase in the acetate concentration in cultures of bacterium V2 growing on a mixture of acetate and ethanol (data not shown), also referring to acetate formation from ethanol.

The pathway proposed (Fig. 4) is also supported by calculations of growth yields, since those with vinyl acetate were similar to the sum of the yields obtained with acetate and acetaldehyde.

The vinyl acetate pathway is cytoplasmic as indicated by the intracellular location of the enzymes involved (Table 2).

The glyoxylate bypass is employed. The acetate derived from vinyl acetate was converted to acetyl-CoA since acetyl-CoA synthetase was active in cells of isolate V2 growing on vinyl acetate (Table 3 and Fig. 4). Subsequently, acetyl-CoA was oxidized by the enzymes of the tricarboxylic acid cycle (Table 3). The presence of isocitrate lyase suggested that the glyoxylate bypass was in operation (Table 3).

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